

What is Claimed is:

1. A method for transforming maize using *Agrobacterium* comprising the steps of:
- contacting at least one immature embryo from a maize plant with *Agrobacterium* capable of transferring at least one gene to the embryo;
  - co-cultivating the embryo with *Agrobacterium*;
  - culturing the embryo in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos expressing the gene; and
  - regenerating plants expressing the gene.
2. The method of Claim 1 wherein the contacting step additionally comprises contacting the immature embryos with *Agrobacterium* in a medium comprising N6 salts.
3. The method of Claim 1 wherein the contacting step additionally comprises contacting the immature embryos with *Agrobacterium* in a medium comprising MS salts.
4. The method of Claim 1 wherein the contacting step takes place in the absence of  $\text{AgNO}_3$ .
5. The method of Claim 1 wherein the immature embryos are preferably from a size of about 0.3 mm to about 4 mm in length.
6. The method of Claim 5 wherein the embryos are preferably about 0.8 mm to about 2.0 mm in length.
7. The method of Claim 1 wherein the concentration of *Agrobacterium* is about  $1 \times 10^8$  cfu/ml to about  $1.5 \times 10^9$  cfu/ml.
8. The method of Claim 7 wherein the *Agrobacterium* concentration is preferably from about  $0.5 \times 10^9$  to about  $1.0 \times 10^9$  cfu/ml.
9. The method of Claim 1 wherein the contacting step takes place in a liquid suspension.

10. The method of Claim 1 wherein the co-cultivation step takes place on a solid medium.
11. The method of Claim 2 wherein a medium containing N6 salts is used in the co-cultivation step.
12. The method of Claim 3 wherein a medium containing MS salts is used in the co-cultivation step.
13. The method of Claim 1 wherein a medium containing MS salts is used in the regeneration step.
14. The method of Claim 2 wherein a medium containing N6 salts is used in the co-cultivation and selection step.
15. The method of Claim 3 wherein a medium containing MS salts is used in the co-cultivation step and a medium containing MS salts is used in the regeneration step.
16. The method of Claim 1 additionally comprising the step of resting the embryos by culturing the embryos in medium containing an antibiotic capable of inhibiting the growth of *Agrobacterium*.
17. The method of Claim 16 wherein the antibiotic is carbenicillin.
18. The method of Claim 16 wherein the antibiotic in the selection step is carbenicillin.
19. The method of Claim 17 wherein the concentration of carbenicillin is about 50 mg/l to about 250 mg/l.
20. The method of Claim 17 wherein the concentration of carbenicillin is about 100 mg/l.
21. The method of Claim 16 wherein the embryos are cultured for about 1 to about 15 days.
22. The method of Claim 16 wherein the embryos are cultured for about 3 to about 5 days.
23. The method of Claim 1 wherein the embryos are cultured in a PHI basic media system.

24. The method of Claim 1 wherein the embryos are cultured in a PHI combined media system.

25. Maize plants transformed by the method of Claim 1.

26. Maize cells transformed by the method of Claim 1.

27. A method for transforming maize using *Agrobacterium* comprising the steps of:

contacting at least one immature embryo from a maize plant with *Agrobacterium* capable of transferring at least one gene to said embryo in a medium comprising N6 salts;

co-cultivating the embryo with *Agrobacterium* in a medium comprising N6 salts;

culturing the embryo in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos expressing the gene; and

regenerating plants expressing the gene in a medium comprising MS salts.

28. The method of Claim 27 wherein the medium of the contacting step lacks  $\text{AgNO}_3$ .

29. The method of Claim 28 wherein the medium of the co-cultivating step includes  $\text{AgNO}_3$ .

30. The method of Claim 27 wherein the *Agrobacterium* concentration is about  $1 \times 10^8$  cfu/ml to about  $1.5 \times 10^9$  cfu/ml.

31. The method of Claim 30 wherein the *Agrobacterium* concentration is about  $0.5 \times 10^9$  to about  $1.0 \times 10^9$  cfu/ml.

32. The method of Claim 27 wherein the contacting step takes place in a liquid and the co-cultivating and culturing steps take place on a solid medium.

33. The method of Claim 27 additionally comprising the step of resting the embryo by culturing the embryo in a medium containing an antibiotic capable of inhibiting the growth of *Agrobacterium*.

34. ~~The method of Claim 33 wherein the antibiotic is carbenicillin.~~
35. ~~Maize plants transformed by the method of Claim 27.~~
36. ~~Maize cells transformed by the method of Claim 27.~~
37. A method for transforming maize using *Agrobacterium* comprising the steps of:
  - contacting at least one immature embryo from a maize plant with *Agrobacterium* capable of transferring at least one gene to said embryo in a medium comprising N6 or MS salts;
  - co-cultivating the embryo with *Agrobacterium* in a medium comprising MS salts;
  - culturing the embryo in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos expressing the gene; and
  - regenerating plants expressing the gene in a medium comprising MS salts.
38. The method of Claim 37 wherein the medium of the contacting step lacks AgNO<sub>3</sub>.
39. The method of Claim 38 wherein the medium of the co-cultivating step includes AgNO<sub>3</sub>.
40. The method of Claim 37 wherein the *Agrobacterium* concentration is about  $1 \times 10^8$  cfu/ml to about  $1.5 \times 10^9$  cfu/ml.
41. The method of Claim 40 wherein the *Agrobacterium* concentration is about  $0.5 \times 10^9$  to about  $1.0 \times 10^9$  cfu/ml.
42. The method of Claim 37 wherein the contacting step takes place in a liquid and the co-cultivating and culturing steps take place on a solid medium.
43. The method of Claim 37 additionally comprising the step of resting the embryo by culturing the embryo in a medium containing an antibiotic capable of inhibiting the growth of *Agrobacterium*.
44. The method of Claim 43 wherein the antibiotic is carbenicillin.

45. Maize plants transformed by the method of Claim 37.
46. Maize cells transformed by the method of Claim 37.
47. A method for optimizing the production of transgenic maize plants of a first genotype using *Agrobacterium*-mediated transformation comprising the steps of:
- isolating immature embryos from maize;
  - separating the embryos into treatment groups;
  - incubating each treatment group separately in a medium comprising N6 or MS salts and in a suspension of *Agrobacterium* at concentrations ranging from about  $1 \times 10^8$  cfu/ml to about  $1 \times 10^{10}$  cfu/ml;
  - co-cultivating the embryos with *Agrobacterium* on a solid medium;
  - culturing the embryos in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos transformed by *Agrobacterium*;
  - identifying the treatment group with the highest transformation frequency ;
- and
- using the concentration of *Agrobacterium* generating the highest transformation frequency to transform other embryos from the first genotype.
48. The method of Claim 47 wherein the medium of the incubating step and the co-cultivating step is a medium comprising N6 salts.
49. The method of Claim 47 wherein the medium of the incubating step is a medium comprising MS salts and the medium of the co-cultivating step is a medium comprising N6 salts.
50. The method of Claim 47 wherein the medium of the incubating step is a medium comprising N6 salts and the medium of the co-cultivating step is a medium comprising MS salts.
51. The method of Claim 47 additionally comprising the step of resting the embryo by culturing the embryo in a medium containing an antibiotic capable of inhibiting the growth of *Agrobacterium*.

00663096-110397

52. The method of Claim 47 wherein the antibiotic is carbenicillin.
53. The method of Claim 47 wherein the combined length of the co-cultivating step and the resting step is at least three days.
54. The method of Claim 51 wherein the length of the resting step is from 0 to about 10 days.
55. The method of Claim 53 wherein the length of the resting step is about 3 to about 5 days.
56. Transformed maize plants produced by a method comprising the steps of:
- contacting at least one immature embryo from a maize plant with *Agrobacterium* capable of transferring at least one gene to the embryo;
  - co-cultivating the embryo with *Agrobacterium*;
  - culturing the embryo in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos expressing the gene; and
  - regenerating plants expressing the gene.
57. Transformed maize cells produced by a method comprising the steps of:
- contacting at least one cell from a maize plant with *Agrobacterium* capable of transferring at least one gene to the cell;
  - co-cultivating the cell with *Agrobacterium*; and
  - culturing the cell in a medium comprising N6 salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for cells expressing the gene: and
  - identifying cells expressing the gene.
58. The method of Claim 57 wherein the cells in the contacting step are obtained from a culture of maize cells.
59. The method of Claim 57 wherein the cells in the contacting step are in an isolated tissue fragment.

60. The method of Claim 59 wherein the tissue fragment is an intact maize embryo.

61. A method for transforming maize using *Agrobacterium* comprising the steps of:

contacting at least one immature embryo from a maize plant with *Agrobacterium* capable of transferring at least one gene to the embryo;  
co-cultivating the embryo with *Agrobacterium*;  
culturing the embryo in a medium containing salts other than MS salts, an antibiotic capable of inhibiting the growth of *Agrobacterium*, and a selective agent to select for embryos expressing the gene; and  
regenerating plants expressing the gene.

26 OCT 1996 11:03 AM

add  
E2